UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

NOV 23 1983

MEMORANDUM

SUBJECT: Re-Review of The 24-Month Feeding/Oncogenicity Study in Mice Using Blazer. Caswell No. 755D

TO: Richard Mountfort, PM #23
Registration Division (TS-767)

THRU: William Butler, Section Head
Toxicology Branch
Hazard Evaluation Division, (TS-767)
and
William Burnam, Branch Chief
Toxicology Branch
Hazard Evaluation Division (TS-769)

Registrant: Rohm-Haas

Background:

Blazer is structurally related to a series of chemicals which have been found to have either teratogenic, oncogenic and/or mutagenic potential (Tok, Goal, Flex, RH-0265, Hoelon, Fusiland and Tackle).

Tackle has the identical chemical structure as Blazer. Blazer which is registered by Rohm-Haas, has established permanent tolerances in or on the following (180.383):

-liver and kidney of cattle, goats, hogs, horses, sheep at 0.02 ppm

-meat, fat, meat byproducts of poultry at 0.02 ppm

-milk and eggs at 0.02 ppm

-soybeans at 0.1 ppm

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RECOMMENDATION:

An oncogenic risk assessment is currently underway because an oncogenic potential was demonstrated in mice being fed Blazer for 24 months (International Research and Development Corp, Report No. 285-013a, dated March 6, 1979). As indicated in the appended review, a statistically significant (Chi-square) increase in liver tumors were observed in the high dose (270/1.25 ppm) females when compared to concurrent water controls. The following table summarizes the liver tumors:

Summary Of Liver Tumors In Mice Fed Blazer (24 months)

	Male Mice				Female Mice			
Dose (ppm)	0 a/	7.5	45.0	270(1.25) b/	0 a/	7.5	45.0	270(1.25) b/
# Livers examined		69	80	70	80	69	80	66
# Livers with carcinoma only	8	12	11	8	1	3	0	3
# Livers with adenoma only	9	3	14	12	5	1	4	11
# Livers with carcinoma and adenoma	2	3	3	7	1	1	0	1
Total # Livers with Tumors	19/79	18/69	28/80	27/70	7/80	5/69	4/80	15/66*

a/ Water control
b/ Animals fed 1.25 ppm until week 17 and then changed to 270 ppm until termination
* P = 0.05

NOTE: CHARLES and TABLE [illegible] CORROBORATED WITH DYNAMAC (11-21-83)

These above data are based on the individual animal data provided in the report and not the summary tables provided in the report, as there appear to be some counting discrepancies. Due to these discrepancies and the critical importance of this study to accurately establish the oncogenic potential of Blazer, a data audit has been formally requested. In the meantime the registrant should be requested to provide (a) either an applicator (mixer/loader/applicator) exposure study or estimates of applicator exposure for each application technique and use, and (b) either a dermal penetration study or estimate of dermal penetration with rationale.

Carolyn Gregorio, Toxicologist CAG 10-6-83
Toxicology Branch/HED (TS-769

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Tox 41:C Gregorio:LM:9/22/83:11745
Revised:DCR-32801:Tox 41:C Gregorio:bje:9/28/83:11745
REVISED:DCR-32804:CBI 5 TOX:C Gregorio:10/5/83:LM

Study Title: 24-Month Feeding/Onco - Mice

Accession Number: Not Reported

MRID Number: Not Reported

Sponsor: Rohm and Haas Company

Contracting Lab: International Research and Development Corporation (Report No. 285-013a)

Date: March 6, 1979

Material: Blazer (Sodium Salt of Acifluoren; RH-6201)
Purity: 39.4-40.5%

Compound Stability: No data were presented in the submitted report with regard to compound stability.

Concentration of Compound in Feed: In an appended report (Report No. TR-34H-77-14) "food samples used in the first year of chronic toxicity studies" were reported. Food analysis was taken on the following dates: 6-9-76, 7-28-76 and 12-1-76. These dates, however, do not correspond to one year, but approximately 6 months (study was initiated on June 9, 1976 and terminated May 18 and 19, 1978). The compound concentration varied acceptably for the 7.5 and 45 ppm levels. The high dose of 270 ppm (changed from 1.25 ppm at week 17) was analyzed only once. Analysis of control diets indicated an average 0.02 ppm of test compound.

In another appended report (Report No. 34H-78-34), samples were taken "from feed used in week 104 of the study." However, the table found in the report provides analyses for "week 1, 4, 8, 26 and 104," (the high dose was only analyzed on week 26 and 104.) Based on the provided analyses, the compound concentration in the feed varied acceptably. Analysis of the control diets indicated an average 0.05 $\pm$ 0.10 ppm of test compound.

Stability of Compound in Feed: No data were presented in the submitted report.

Protocol: Eight hundred Charles River CD-1 mice obtained from Charles River Breeding Labs (Wilmington, Massachusetts) were "arbitrarily placed into temporary groups, as they were removed from shipping crates, and assigned temporary numbers." The animals, 80 males (weighing 24-39 grams) and 80 females (weighing 20-33 grams) were assigned to each dose group. Blazer was given in the diet at concentrations of 0, 1.25 [changed to 270 ppm at week 17], 7.5 and 45 ppm.

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The diets were prepared in the following manner: "RH-6201 was dissolved in a small quantity of distilled water to facilitate mixing with the basal diet (ground Purina Laboratory Chow). Two control groups of 80 mice/sex/group were fed basal diet mixed with similar quantities of either acetone or distilled water." It should be noted that the report states (section labeled: II. Compound, page 9) that the compound is a "brown, slightly viscous liquid." "The appropriate amount of RH-6201 was mixed with 7 ml distilled water and premixed with 500 grams basal diet using a Hobart food mixer." Then the premix was added and mixed with additional basal diet "to yield 8 kg of prepared test diet. Control diets were prepared by the addition of 7 ml of ACS grade acetone or distilled water to basal diet to provide an identical quantity of premix as for treated groups." Water and test diets were available ad libitum. For purposes of this review, the water control was used for all comparisons since it was the substance mixed in the diets.

"The mice were housed individually in hanging wire-mesh cages and were maintained in a temperature, humidity and light controlled room."

General Observations: All mice wre observed daily for overt signs of toxicity, morbundity, and mortality.

Body Weights and Food Consumption: Individual body weights and "sex-group" food consumption were recorded weekly for the first 13 weeks and monthly thereafter.

Hematology: Blood samples were obtained from 10 animals/sex/dose at 3, 12, 18 and 24 months. Hematological studies included: hemoglobin, hematocrit, total erythrocyte count, total and differential leucocyte counts, clotting times prothrombin time, and cell morphology were done at 3, 12 and 18 months and termination. "At 18 months, formation of slides for differential count revealed a large number of platelets. These were recorded as increased (inc) where applicable."

Biochemistry: Blood samples were obtained from 10 animals/sex/dose at 3, 12, 18 and 24 months. Biochemical studies included: fasting glucose, blood urea nitrogen (BUN), total protein, albumin, glutamic pyruvic transamine activity (SGPT), alkaline phosphatase activity, globulin, and the albumin:globulin ratio. Serum oxaloacetic transaminase activity (SGOT) was inadvertently determined at 12 months of study only.

Pathology:

Gross Pathology: Complete necropsy was conducted on terminally sacrificed animals (supervised by Dr. Curt Barthel, consultant veterinary pathologist). "Fixed tissues of terminally sacrificed mice and mice that died or were moribund from 12

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months to termination were sent to Histolabs, Inc. for routine histological processing, then sent to Dr. Bartel for microscopic examination."

"Fixed tissues of mice sacrificed at 12 months and mice which died or were sacrificed in extremis during the first 12 months of the study were delivered to American Histolabs, Inc. (Silver Spring, Maryland) for histological processing, then returned to IRDC for microscopic examination."

Organ Weights: The weights of the following organs were recorded: spleen, liver, kidneys, brain, heart, thyroid, adrenals, testes/ovary.

Histopathology:

a. 3 Month Interim Sacrifice: The following tissues were examined from 10 animals/sex/dose "in both control groups and the 45 ppm dosage level" (asterisk denotes those tissues examined from the 1.25 [changed to 270 ppm at week 17] and 7.5 ppm doses):

aorta	liver	spleen*
adrenals	lungs	stomach
bone marrow*	mandibular lymph glands	thyroid
brain	mammary gland	urinary bladder
eye	pancreas	uterus/prostate
gallbladder	salivary gland	
gonads	skin	
heart	spinal cord	
small intestine	pituitary	gross lesions
large intestine	skeletal muscle with nerve	tissue masses and tumors
kidneys		

b. 12 Month Interim Sacrifice: The following tissues were examined from 10 animals/sex dose "in the control groups, the 270 (1.25) and 45 ppm dosage levels" (asterisk denotes those tissues examined from the 7.5 ppm dose):

Spleen and bone marrow were microscopically examined from mice at the 7.5 and 1.25 ppm dosage levels. In addition, special stains for iron were performed on tissues of selected mice at the 45.0 ppm dosage level.

At the 12 month interim sacrifice, the following tissues from each mouse in the control groups, the 270 (1.25) and 45.0 ppm dosage levels were embedded in paraffin, stained with hematoxylin and eosin, and examined microscopically:

adrenals*	kidneys	spleen*
bone marrow*	liver*	thyroid/ parathyroid*
brain	lung	
gonads*	mandibular lymph glands*	
heart with coronary vessels	pancreas*	any other gross lesions*
	pituitary*	

c. Terminal Sacrifice: The Protocol Section of the report does not state which tissues were to be examined in which dose groups.

Statistics: "All statistical analyses compared treatment groups with each of the control groups by sex. Body weights at 13, 26, 39, 52, 66, 78, 91 and 100, and hematological and biochemical parameters (with the exception of SGOT) at 3, 12 and/or 18 months and termination were compared by analysis of variance (one-way classification), Bartlett's test for homogeneity of variances, and the appropriate t-test (for equal and unequal variances) as described by Steel and Torrie. Dunnett's multiple comparison tables were used to judge significance of differences.

Results:

General Behavior: The authors reported, "The following observations were noted for both control and treated mice: an accumulation of yellow material in the anogenital region; pale color to skin and/or eyes; ocular changes including corneal opacity and eccentric pupils; and moribundity usually preceded by tremors, labored breathing and an apparent reduction in body heat. A slightly higher frequency of these observations, particularly the yellow material in the anogenital region and paling of eyes and/or skin, was noted for the treated mice predominantely males, although the incidence of these signs was greatest for mice in the 45- and 270-ppm dosage levels. The small numerical differences between these two groups and mice treated at the lowest dosage level precludes a definitive conclusion as to a dosage-related effect. The incidence of these signs in the treated males versus the control males is indicative of probable compound effect. Incidental findings included: tonic convulsions, circling, swelling of extremities or body, and red, extended penis or red material at the vaginal openings."

Mortality: Similar mortality rates were observed for all groups. Survival was as follows:

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Treatment Level	No. Surviving/No. Initiated* Male	Females
Control (acetone)	21/55	27/60
Control (distilled water)	27/58	32/60
7.5 ppm	26/60	23/59
45.0 ppm	23/59	28/60
270 (1.25) ppm	31/60	21/60

*Interim sacrifices not included.

Body Weights: Mean body weights were similar for all groups throughout the study.

Food Consumption: Although the mean food consumption appears to be similar for all groups throughout the study, explanation of the Table 4 is needed. It is not clear what "grams/ kilograms/ day" and "mcg/kg/day" represent.

Hematology: Mean hematological values were similar for all groups throughout the study.

Biochemistry: Mean biochemical values were similar for all groups throughout the study with the exception of alkaline phosphatase (AP) and serum glutamic pyruvic transaminase (SGPT) activity.

The following table demonstrates the apparent dose related elevation of alkaline phosphatase and SGPT in males at the 45 and 270 (1.25 ppm) doses. However, due to the variability of the standard deviation for these parameters, statistical analysis was not performed. NOTE: A series of statistical tests will be performed during the conduct of the oncogenic risk assessment.

Mean Alkaline Phosphatase and SGPT Values

	Males					Females				
Dose (ppm)	Control (Acetone)	Control (Water)	270(1.25)*	7.5	45.0	Control (Acetone	Control (Water)	270 (1.25)*	7.5	45.0
Alkaline Phosphatase										
- 12 Month	47±26.4	82±76.1	95±42.7	61±26.6	69±42.8	100±49.6	83±27.4	113±63.4	111±46.7	105±30.5
- 24 Month	132±125.9	85±44.8	347±355.4	77±39.9	169±194.8	122±48.6	179±105.3	122±81.5	140±95.8	111±46.4
SGPT										
- 12 Month	71±33.5	95±35.4	146±54.7	105±37.8	107±52	72±21.2	74±31.9	174±51.0	88±24.6	142±65.9
- 24 Month	43±20.7	37±17.1	169±165.5	62±27	61±35.5	60±35.2	31±14.4	42±20.6	40±18.7	52±20.4

*Dose increased from 1.25 ppm to 270 ppm at week 17.

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Organ Weights:

a. 3-Month Sacrifice: Organ-to-bodyweight ratio for spleen, liver, kidneys, testes/ovaries, heart, thyroid and adrenals were similar for all groups.

b. 12-Month Sacrifice: A dose-related increase in absolute and relative (% body weight) weights of the liver and kidneys were observed in treated males.

Mean Absolute (G) and Relative (% Body Weight) for Liver - Males

Dose (PPM)	Control (Acetone)	Control (Water)	270(1.25)*	7.5	45.0
Bodyweight(G)	36	38	37	37	38
- Liver					
Absolute(G)	1.53	1.54	1.76	1.64	1.72
Relative(%)	4.24	4.11	4.73	4.44	4.55

*Dose increased from 1.25 ppm to 270 ppm at week 17.

c. Terminal Sacrifice: A dose-related increase in absolute and relative (% body weight) weights were observed in the liver and kidneys in treated males.

Mean Absolute (G) and Relative (% Body Weight) for Liver, Kidneys - Males

Dose (PPM)	Control (Acetone)	Control (Water)	270(1.25)*	7.5 ppm	45.0
Bodyweight(G)	32	32	31	31	32
- Liver					
Absolute(G)	1.98	1.85	2.40[a,b]	2.03	2.08
Relative(%)	6.23	5.86	7.65[a,b]	6.41	6.58
- Kidneys					
Absolute(G)	0.842	0.843	0.937	0.832	0.837
Relative(%)	2.66	2.66	3.00[a,c]	2.68	6.58

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*Dose increased from 1.25 ppm to 270 ppm at week 17.
a/ = Significantly different from acetone control, p <0.05.
b/ = Significantly different from water control, p <0.01.
c/ = Significantly different from water control, p <0.05.

Gross Pathology: Gross masses/nodules of the liver were reported as follows:

Summary of Liver Masses/Nodules Seen Grossly

	Control (Acetone)	Control (Water)	270/1.25* ppm	7.5 ppm	45.0 ppm
Males					
- 0-12 mo. Sacrifice	0	0	1	4	0
- Early Deaths	6	4	6	6	10
- Final Sacrifice	5	6	13	6	9
# Livers Examined	61	78	70	69	80
Total	11/61	10/78	20/70	16/69	19/80
Females					
- 0-12 mo. Sacrifice	0	0	0	1	0
- Early Deaths	2	0	5	3	2
- Final Sacrifice	4	2	8	4	0
# Lives Examined	71	79	66	69	80
Total	6/71	2/79	13/66	8/69	2/80

*Dose increased from 1.25 ppm to 270 ppm at week 17.

Histopathology: A statistically significant (Chi-square) increase in liver tumors were observed in the high dose (270/1.25 ppm) females when compared to concurrent water controls. The following table summarizes the liver tumor incidence:

Summary of Liver Tumors in Mice Fed Blazer (24 Months)

DOSE (PPM)	MALES 0	7.5	45.0	270(1.25) a/	FEMALES 0	7.5	45.0	270(1.25) a/
3 Month Sacrifice								
# Livers Examined	10	0	10	0	10	0	10	0
- Carcinoma (only)	0	-	0	-	0	-	0	-
- Adenoma (only)	0	-	0	-	0	-	0	-
- Carcinoma & Adenoma	0	-	0	-	0	-	0	-
TOTAL - 3 MONTHS	0/10	0	0/10	0	0/10	0	0/10	0
12 Month Sacrifice								
# Livers Examined	12	10	11	10	10	9	7	7
- Carcinoma (only)	0	3	0	0	0	0	0	0
- Adenoma (only)	0	0	0	1	0	0	0	0
- Carcinoma & Adenoma	0	0	0	0	0	0	0	0
TOTAL - 12 MONTHS	0/12	3/10	0/11	1/10	0/10	0/9	0/7	0/7
EARLY DEATHS 0-12 MONTHS								
# Livers Examined	3	7	5	3	9	7	10	6
- Carcinoma (only)	0	0	0	0	0	0	0	0
- Adenoma (only)	0	0	0	0	0	0	0	0
- Carcinoma & Adenoma	0	0	0	0	0	0	0	0
TOTAL - EARLY DEATHS (3-12 MONTHS)	0/3	0/7	0/5	0/3	0/9	0/7	0/10	0/6
EARLY DEATHS 12-24 MONTHS								
# Livers Examined	26	26	31	26	23	31	31	37
- Carcinoma (only)	5	4	7	4	1	0	0	1
- Adenoma (only)	2	0	7	3	1	0	1	4
- Carcinoma & Adenoma	0	1	0	2	0	0	0	0
TOTAL - EARLY DEATHS (12-24 MONTHS)	7/26	5/26	14/31	9/26	2/23	0/31	1/31	5/37
TERMINAL SACRIFICE- 24 MONTHS								
# Livers Examined	28	26	23	31	28	22	22	16
- Carcinoma (only)	3	5	4	4	0	2	0	2
- Adenoma (only)	7	2	7	8	4	2	3	7
- Carcinoma & Adenoma	2	3	3	5	1	1	0	1
TOTAL - TERMINAL SACRIFICE	12/28	10/26	14/23	17/31	5/28	5/21	3/22	10/16
TOTALS								
# Livers Examined	79	69	80	70	80	69	80	66
- Carcinoma (only)	8	12	11	8	1	2	0	3
- Adenoma (only)	9	5	14	12	5	2	4	11
- Carcinoma & Adenoma	2	3	3	7	1	1	0	1
TOTAL	19/79	18/69	28/80	27/70	7/80	5/69	4/80	15/66*

a/ Animals fed 1.25 ppm until week 17 and then changed to 270 ppm until term.

*P = 0.05.

NOTE: CHANGES ON TABLE CORROBORATED WITH DYNAMAC (11-21-83)

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Note: Statistical calculations were done with the "water" control since "for each treated group, the appropriate amount of RH-620: [Blazer] was mixed with 7 ml distilled water...."

In the submitted report, the summary table provided for "selected histopathologic findings in the liver from 3 months through termination" does not agree with the individual animal data submitted in the report, as seen in the following table:

Total Numer Livers Examined

Dose (PPM)	Acetone	Water	7.5	45.0	270 (1.25)
Males					
- Registrant Count	70	69	70	70	70
- Reviewer Count	61	78	69	80	70
Females					
- Registrant Count	69	70	69	70	69
- Reviewer Count	71	79	69	80	66

This type of noted discrepancy does impede the assessment of the oncogenic potential of this compound. In order to clarify this issue, a data audit has been formally requested (memo W. L. Burnam to J. G. Touhey, Dated 9-9-83).

Conclusion: Based on the presented data, an oncogenic potential was demonstrated in mice being fed Blazer for 24 months. A statistically significant (Chi-square) increase in total liver tumors was observed in the high dose (270/1.25 ppm) females when compared to concurrent water controls. Further resolution of counting discrepancies (total number livers examined) is necessary. A data audit has been formally requested. In addition, an oncogenic risk assessment is currently underway.

Classification: Supplementary (resolution of histopatholgy questions; explanation of why the 270 ppm diet was analyzed only once; provide stability of the compound in feed data. Explanation of Table 4 (Food Consumption).

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DCR-11746:C.Gregorio:TOX.42:9/22/83:pjb
REVISED-9/28/83:DCR-32802:TOX-42:efs
REVISED:DCR-32805:CBI 5 TOX:10/5/83:LM

END